C10 1. (amended) An isolated protein comprising a portion of SEQ ID NO:2, wherein said portion is selected from the group consisting of residues 33-75, residues 93-157, residues 299-351, and residues 412-548.

C11 7. (twice amended) The isolated protein of claim 1, wherein said portion is selected from the group consisting of residues 93-157, residues 299-351, and residues 412-548.

23. (twice amended) A protein produced by a method comprising the steps of:

(a) culturing a cell comprising an expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA segment encoding a protein comprising a portion of SEQ ID NO:2, wherein said portion is selected from the group consisting of residues 33-75, residues 93-157, residues 299-351, and residues 412-548; and

a transcription terminator

C12 under conditions whereby the DNA segment is expressed; and

(b) recovering the protein encoded by the DNA segment.

24. (twice amended) The protein of claim 23 wherein said portion is selected from the group consisting of residues 93-157, residues 299-351, and residues 412-548.

Please cancel claim 8.

REMARKS

Reconsideration of the application in view of the above amendments and following remarks is requested. Claims 1-7, 23, 24, and 26 are now in the case. Claims 1, 7, 23, and 24 have been amended. Claim 8 has been cancelled. No new matter has been added.

Claims 1, 7, 23, and 24 have been amended, and claim 8 has been cancelled, solely for business reasons. Applicants wish to expedite issuance of claims drawn to subject matter of commercial importance. Applicants reserve the right to prosecute claims to cancelled subject matter in one or more continuing applications.

The specification has been amended to recite trademarks in capital letters.

Amended paragraphs and claims, marked to show changes, are included in the enclosed Appendix.

Claim 8 was rejected under 35 U.S.C. § 112, second paragraph on the grounds of being indefinite in the recitation of "at least." This rejection is believed to be obviated by the cancellation of claim 8 for other reasons. However, Applicants respectfully submit that claim 8 complies with the requirements of the second paragraph of § 112. The claim is clearly open-ended with regard to the contiguous amino acid residues of SEQ ID NO:2, both in the recitation of "at least" and in the use of the transitional term "comprising."

Claims 1-2, 6-7, 23-34, and 26 stand rejected under 35 U.S.C. § 112, first paragraph. The Office believes that the specification "does not reasonably provide enablement for polypeptides comprising the immunoglobulin domain of the polypeptide of SEQ ID NO:2."

This rejection is believed to be obviated by the amendment of claims 1, 7, 23, and 24 for other reasons. Applicants respectfully submit, however, that the rejected claims are fully enabled by the specification as filed. Activities of the immunoglobulin domain are set forth within the specification at page 10 and elsewhere. As disclosed at pages 32-34, this region of the protein could be used as an immunogen to raise antibodies that could be used, *inter alia*, for purification of zkun6 proteins. As shown in Figs. 2E-2D, residues 203-286 of SEQ ID NO:2 include several hydrophilic regions, including residues 219-234. No undue experimentation would be required to make and use a polypeptide comprising residues 203-286 of SEQ ID NO:2.

Claim 8 stands rejected under 35 U.S.C. § 112, first paragraph. The Office believes that the claim contains "subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention."

This rejection is believed to be obviated by the cancellation of claim 8 for other reasons. However, Applicants respectfully submit that claim 8 fully complies with the requirements of the first paragraph of § 112. Applicants have disclosed the complete sequence of a human zkun6 protein. Applicants have further disclosed a variety of fusion proteins, for example at pages 15-16 of the specification. Fusion protein technology is well known and widely used in the art. Thus, one of skill in the art can readily envisage polypeptide sequences that include the recited portions of SEQ ID NO:2 because those portions can be fused to known sequences. Although there may be substantial variability among the species of polypeptides encompassed within the scope of the claim, the necessary common attribute is the at least 15 contiguous amino acid residues recited in

the claim. One skilled in the art would recognize from Applicants' disclosure that Applicants were in possession of the genus of polypeptides recited in claim 8.

Claim 8 also stands rejected under 35 U.S.C. § 112, first paragraph on the grounds that the specification allegedly does not "provide enablement for any polypeptide comprising at least 15 contiguous amino acids of SEQ ID NO:2."

This rejection is believed to be obviated by the cancellation of claim 8 for other reasons. However, Applicants respectfully submit that claim 8 fully complies with the requirements of the first paragraph of § 112. Applicants have disclosed the complete sequence of a human zkun6 protein. Applicants have further disclosed a variety of fusion proteins, for example at pages 15-16 of the specification. Fusion proteins are useful in the production of antibodies as disclosed at page 33 of the specification. Fusion protein technology is well known and widely used in the art, including use in antibody production. Fusion proteins can include cleavage sites (page 16), which would allow separation of an immunogenic sequence from a fusion partner if, *arguendo*, folding of the fusion protein masked the immunogenic portion. Applicants have disclosed both how to make and how to use the polypeptides recited in claim 8.

Applicants believe that each rejection has been addressed and overcome. Reconsideration of the application and its allowance are requested. If for any reason the Examiner feels that a telephone conference would expedite prosecution of the application, the Examiner is invited to telephone the undersigned at (206) 442-6673.

Respectfully Submitted,

Gary E. Parker
Registration No. 31,648

Enclosures:
- Appendix
- Amendment Fee Transmittal (in duplicate)
- Postcard

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Appendix

Please replace the paragraph at page 25, line 25 through page 26, line 11 with the following amended paragraph:

Cultured mammalian cells are suitable hosts for use within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler et al., *Cell* 14:725, 1978; Corsaro and Pearson, *Somatic Cell Genetics* 7:603, 1981: Graham and Van der Eb, *Virology* 52:456, 1973), electroporation (Neumann et al., *EMBO J.* 1:841-845, 1982), DEAE-dextran mediated transfection (Ausubel et al., ibid.), and liposome-mediated transfection (Hawley-Nelson et al., *Focus* 15:73, 1993; Ciccarone et al., *Focus* 15:80, 1993). The production of recombinant polypeptides in cultured mammalian cells is disclosed by, for example, Levinson et al., U.S. Patent No. 4,713,339; Hagen et al., U.S. Patent No. 4,784,950; Palmiter et al., U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Suitable cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), 293 (ATCC No. CRL 1573; Graham et al., *J. Gen. Virol.* 36:59-72, 1977) and Chinese hamster ovary (e.g. CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the [American Type Culture Collection] AMERICAN TYPE CULTURE COLLECTION, 10801 University Boulevard, Manassas, VA. Suitable promoters include those from metallothionein genes (U.S. Patent Nos. 4,579,821 and 4,601,978), SV-40, cytomegalovirus (U.S. Patent No. 4,956,288), and the adenovirus major late promoter. Expression vectors for use in mammalian cells include pZP-1 and pZP-9, which have been deposited with the [American Type Culture Collection] AMERICAN TYPE CULTURE COLLECTION, 10801 University Boulevard, Manassas, VA under accession numbers 98669 and 98668, respectively, and derivatives thereof.

Please replace the paragraph at page 34, line 30 through page 35, line 9 with the following amended paragraph:

A panel of cDNAs from human tissues was screened by PCR for zkun6 expression. The panel included 77 cDNA samples from various normal and cancerous human tissues and cell lines as shown in Table 5. The panel was set up in a 96-well format that included a human genomic DNA ([Clontech] CLONTECH Laboratories,

Inc., Palo Alto, CA) positive control sample. Each well contained approximately 0.2-100 pg/µl of cDNA. The PCR reaction mixtures contained oligonucleotide primers ZC28,995 (SEQ ID NO:8) and ZC28,996 (SEQ ID NO:9), *Taq* DNA polymerase ([ExTaq™] EXTAQ; TAKARA Shuzo Co. Ltd., Biomedicals Group, Japan), and a density increasing agent and tracking dye ([RediLoad™] REDILOAD, [Research Genetics] RESEARCH GENETICS, Inc., Huntsville, AL). The reaction mixtures were incubated at 94°C for 2 minutes; followed by 35 cycles of 94°C for 30 seconds, 61.4°C for 30 seconds, and 72°C for 30 seconds; followed by a 5-minute incubation at 72°C. About 10 µl of each of the PCR reaction products was electrophoresed on a 4% agarose gel. The predicted DNA fragment size of ~110 bp was observed in brain, prostate, spinal cord, thyroid, fetal brain, placenta, salivary gland, testis, bone marrow, and stomach tumor, and possibly in islet, kidney, and HaCat cells.

Please replace the paragraph at page 35, lines 10-14 with the following amended paragraph:

The DNA fragments for brain, prostate, fetal brain, and genomic DNA were excised and purified using a commercially available gel extraction kit (obtained from [Qiagen] QIAGEN, Valencia, CA) according to the manufacturer's instructions. Fragments from fetal brain and genomic DNA were confirmed to be human zkun6 DNA by sequencing.

Please replace the paragraph at page 37, lines 3-30 with the following amended paragraph:

The second-round positive pool was plated and transferred to nylon membrane filters ([Hybond-N™] HYBOND-N; Amersham Pharmacia Biotech, Piscataway, NJ). Four filters at approximately 1000 colonies each were prepared. The filters were marked with a hot needle for orientation, then denatured for 6 minutes in 0.5 M NaOH and 1.5 M Tris-HCl pH 7.2. The filters were then neutralized in 1.5 M NaCl and 0.5 M Tris-HCl pH 7.2 for 6 minutes. The DNA was affixed to the filters using a UV crosslinker ([Stratalinker®] STRATALINKER; [Stratagene] STRATAGENE, La Jolla, CA) at 1200 joules. The filters were prewashed at 65°C in prewash buffer (0.25 x SSC, 0.25% SDS, 1mM EDTA). The solution was changed a total of three times over a 45-minute period to remove cell debris. Filters were prehybridized overnight at 65°C in 25 ml of a commercially available hybridization solution ([Expresshyb™] EXPRESSHYB; [Clontech] CLONTECH Laboratories, Inc., Palo Alto, CA.). A probe was generated by PCR using oligonucleotide primers ZC29,898 (SEQ ID NO:10) and ZC29,899 (SEQ ID NO:11), a positive clone from the fetal brain library as template, an

annealing temperature of 76.0°C, and 35 cycles. The resulting PCR fragment was gel purified using a commercially available kit ([QIAquick™] QIAQUICK gel extraction kit; [Qiagen] QIAGEN). The probe was radioactively labeled with ^{32}P using a commercially available kit ([Rediprime™] REDIPRIME II random-prime labeling system; Amersham Pharmacia Biotech) according to the manufacturer's specifications. The probe was purified using a push column ([NucTrap®] NUCTRAP; [Stratagene] STRATAGENE Cloning Systems, La Jolla, CA). Hybridization took place overnight at 65°C in a commercially available hybridization solution ([Expresshyb™] EXPRESSHYB; [Clontech] CLONTECH Laboratories, Inc.). Filters were rinsed four times at 65°C in pre-wash buffer, then exposed to film for 3 days at -80°C. There were 6 positives on the filters. Six clones were picked from the positive areas and streaked out. Ninety-five individual colonies from these six positives were screened by PCR using oligonucleotide primers ZC29,898 (SEQ ID NO:10) and ZC29,899 (SEQ ID NO:11) and an annealing temperature of 61.0°C. Two positives were obtained. One clone (designated clone #1) was sequenced and found to include the 3' end and a sequence corresponding to the gap between the original ESTs.

Please replace the paragraph at page 37, line 31 through page 38, line 6 with the following amended paragraph:

To construct a full-length zkun6 cDNA, DNA was prepared from clone #1 and EST2906640 by the mini-prep method using a commercially available kit (obtained from [Qiagen] QIAGEN). A 1015-bp 5'-end fragment was generated by digesting EST2906640 with EcoRI and AatII. A 1085-bp 3'-end fragment was generated by digesting clone #1 with AatII and XbaI. The two fragments were ligated to plasmid pZP-9, which had been digested with EcoRI and XbaI. The ligation mixture was transformed into *E. coli* strain DH10B™ (obtained from [Life Technologies] LIFE TECHNOLOGIES, Inc., Gaithersburg, MD) by electroporation. Ten clones were picked and checked by PCR using oligonucleotide primers ZC28,995 (SEQ ID NO:8) and ZC28,996 (SEQ ID NO:9) with an annealing temperature of 61.4°C. All clones were positive for the expected ~110-bp band. One clone was sequenced and confirmed to encode human zkun6.

Please replace the paragraph at page 38, lines 12-17 with the following amended paragraph:

11-day and 15-day mouse embryo cDNAs were screened for zkun6 by PCR using oligonucleotide primers ZC37,161 (SEQ ID NO:12) and ZC37,160 (SEQ ID NO:13) and *Taq* DNA polymerase ([ExTaq™] EXTAQ DNA polymerase; [TaKaRa]

TAKARA Biomedicals) plus antibody. The reactions were run at an annealing temperature of 62.8°C with an extension time of 30 seconds for a total of 35 cycles. Products of both reactions were positive.

Please replace the paragraph at page 38, lines 18-27 with the following amended paragraph:

The mouse 15-day embryo library was screened for a full-length clone. This library was an arrayed library representing 9.6 x 10^5 clones made in the vector pCMVSPORT2 ([Life Technologies] LIFE TECHNOLOGIES, Gaithersburg, MD). A working plate containing 80 pools of 12,000 colonies each was screened by PCR using oligonucleotide primers ZC37,161 (SEQ ID NO:12) and ZC37,160 (SEQ ID NO:13) with an annealing temperature of 62.8°C for 35 cycles. There were 3 positives. Pools corresponding to positive pools from the working plate were screened by PCR using the same reaction conditions. Four positives was obtained. Corresponding pools from the original source plates were then screened by PCR using the same reaction conditions. Reaction products were sequence and determined to represent mouse zkun6 DNA.

Please replace the paragraph at page 38, line 30 through page 39, line 7 with the following amended paragraph:

A mammalian expression vector was constructed with the dihyrofolate reductase gene selectable marker under control of the SV40 early promoter, SV40 polyadenylation site, a cloning site to insert the gene of interest under control of the mouse metallothionein 1 (MT-1) promoter, and the human growth hormone (hGH) gene polyadenylation site. The expression vector was designated pZP-9 and has been deposited at the [American Type Culture Collection] AMERICAN TYPE CULTURE COLLECTION, 10801 University Boulevard, Manassas, VA under Accession No. 98668. To facilitate protein purification, the pZP-9 vector was modified by addition of a tissue plasminogen activator (t-PA) secretory signal sequence (see U.S. Patent No. 5,641,655) and a Glu-Glu tag sequence (SEQ ID NO:4) between the MT-1 promoter and hGH terminator. The t-PA secretory signal sequence replaces the native secretory signal sequence for DNAs encoding polypeptides of interest that are inserted into this vector, and expression results in an N-terminally tagged protein. The N-terminally tagged vector was designated pZP9NEE.

Please replace the paragraph at page 39, lines 8-21 with the following amended paragraph:

To construct an expression vector for zkun6 or a portion thereof, PCR is performed on cDNA prepared as disclosed above. Primers are designed such that the PCR product will encode the desired polypeptide (e.g., an intact Kunitz domain or a multi-domain polypeptide) with restriction sites Bam HI in the sense primer and Xho I in the antisense primer to facilitate subcloning into an expression vector. 5 µl of 1/100 diluted cDNA, 20 pmoles of each oligonucleotide primer, and 1 U of a 2:1 mixture of [ExTaq™] EXTAQ DNA polymerase ([TaKaRa] TAKARA Biomedicals) and *Pfu* DNA polymerase ([Stratagene] STRATAGENE, La Jolla, CA) (*ExTaq/Pfu*) are used in 25-µl reaction mixtures. The mixtures are incubated at 94°C for 2 minutes; 3 cycles of 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 30 seconds; 35 cycles of 94°C for 30 seconds, 68°C for 30 seconds; and a 7-minute incubation at 72°C. The PCR product is gel purified and restriction digested with Bam HI and Xho I overnight. The vector pZPNEE is digested with Bam HI and Xho I, and the zkun6 fragment is inserted. The resulting construct is confirmed by sequencing.

Please replace claims 1, 7, 23, and 24 with the following amended claims:

1. (amended) An isolated protein comprising a portion of SEQ ID NO:2, wherein said portion is selected from the group consisting of residues 33-75, residues 93-157, [residues 203-286,] residues 299-351, and residues 412-548.

7. (twice amended) The isolated protein of claim 1, wherein said portion is selected from the group consisting of residues 93-157, [residues 203-286,] residues 299-351, and residues 412-548.

23. (twice amended) A protein produced by a method comprising the steps of:

(a) culturing a cell comprising an expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA segment encoding a protein comprising a portion of SEQ ID NO:2, wherein said portion is selected from the group consisting of residues 33-75, residues 93-157, [residues 203-286,] residues 299-351, and residues 412-548; and

a transcription terminator

under conditions whereby the DNA segment is expressed; and

(b) recovering the protein encoded by the DNA segment.

24. (twice amended) The protein of claim 23 wherein said portion is selected from the group consisting of residues 93-157, [residues 203-286,] residues 299-351, and residues 412-548.